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ABSTRACT

The problem of marking post-larval flounder (*Paralichthys* spp.) with radioisotopes was approached in two ways: (1) by marking with radioactive cerium and cobalt, and (2) by increasing the concentration of stable cobalt in the fish and then making it radioactive through exposure to neutrons in a nuclear reactor (activation analysis).

In the first approach, both cerium 144 and cobalt 60, introduced into the food or water, were used satisfactorily as marks for post-larval flounder. The radioactivity obtained from the water was retained longer than that obtained from food. The amount of cerium accumulated by the fish in 24 hours did not increase when the concentration of cerium 144 was raised from 0.4 to 1.0 μc/ml of sea water, but the amount of cobalt 60 accumulated increased when the concentration was raised from 0.01 to 1.0 μc/ml of sea water.

In the second approach, fish were marked by holding them for 24 hours in sea water with different concentrations of stable cobalt. Fish held 24 hours in sea water containing 15.62 μg of stable cobalt per ml of sea water and then held for 36 days in flowing sea water contained enough stable cobalt to be identified in the laboratory by activation analysis.

INTRODUCTION

The best method of gathering information concerning certain species of fish is to mark, release, and recapture a portion of the population. Techniques such as attaching tags or clipping fins are commonly used for marking large fish, but are unsuitable for small post-larvae. Larval fish have been marked by injection of biological stains or dyes intramuscularly (Dunn and Coker, 1951) and subcutaneously (Wigley, 1952). Injection, however, requires handling of individual fish which may result in injury. In addition, the obvious discoloration of an injected or dyed fish may make it more susceptible to predators.

The recent development of radioisotope techniques has given fishery biologists a new method of marking larval fish which eliminates many of the disadvantages of the methods mentioned above. Scott (1961) found that injections of either iron 59 or iron 55 could be used as a mark for fish over 8 cm long. To mark smaller fish, a method of introducing the radioisotope through food was required, since iron will not stay in solution in water and injection would harm the fish. The use of radioactive cesium 137 as a mark was tested in lamprey ammocoetes and other fish (Scott, 1962). The 47-day effective half-life which was calculated indicated that cesium 137 could be used for short periods. Russian scientists (Kirpichnikov, Svetovidov, and Troshin, 1958; Bogoiavlenskaia and Karzinkin, 1958; Shekhanova, 1958; Bogoiavlenskaia, 1959; Karzinkin, Soldatova, and Shekhanova, 1959) established that calcium 45 can be detected in fish from 1½ to 2 years after marking, but that phosphorus 32 remains useful as a mark for only 2½ to 3 months. The disadvantage of calcium 45 and phosphorus 32 is that both emit beta radiation, which is more difficult to detect than gamma radiation.

This paper describes two methods for marking post-larval flounder (*Paralichthys* spp.): (1) marking with ratioactive cerium and cobalt, and (2) marking with stable cobalt which was subsequently detected by activation analysis.

MATERIALS AND METHODS

Three species of flounder, *Paralichthys dentatus*, *P. albigutta*, and *P. lethostigma*, were collected with a 1-mm-mesh net, one meter in diameter as they migrated into the Newport River estuary near Beaufort, North Carolina. The species were not separated in the experiments.

The radioisotopes used were obtained from

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[2] Based on part of a Master's thesis submitted to North Carolina State University, Raleigh, North Carolina.

TABLE 1.—*Summary of cerium 144 experiments*

Experiment number	Number of fish marked	Average weight (mg)	Method of marking	Concentration of isotope in water (μc/ml)	Marking time (hours)	Days in flowing sea water	Mean cpm/fish at end of experiment
1	6	26	Water	0.002	24	1	0
2	10	41	Water	0.400	24	33	344
3	15	85	Water	1.000	24	50	110
4	10	49	Food	0.400	24	32	68

the Oak Ridge National Laboratory, Oak Ridge, Tennessee, and were diluted to obtain the desired concentration of radioactivity. Specific concentrations are given with the description of each experiment. The stable cobalt was cobalt (ous) chloride ($CoCl_2 \cdot 6H_2O$).

Three detection systems were used to radioassay the flounder: (1) an underwater Beta-Gamma monitor and a 3-inch-diameter crystal with a low-noise multiplier phototube (a portable system that could be used in a field experiment); (2) a decade scaler and a scintillation well counter, set up in the laboratory as a reserve for the portable detector; and (3) a 512-channel analyzer with a 4- by 4-inch NaI (Tl) crystal mounted on a 3-inch phototube and a large shield (inside dimensions $2 \times 2 \times 3$ feet), for identification of activation products.

Both food and water were used to introduce the mark into the fish. Food organisms were held for a fixed period of time in water containing the desired concentration of isotope, rinsed in nonactive water, and fed to the fish. Fish were allowed to feed for either 24 or 48 hours, then removed and placed in flowing water. Fish also were marked by placing them in water containing a predetermined amount of the element, and leaving them for either 24 or 48 hours to accumulate the element; they were then rinsed in nonactive sea water and placed in glass vials containing 5 ml of sea water for radioassay. Fish were assayed individually for 3 min or until 10,000 counts were obtained, whichever occurred first. The size of the vial restricted the flounder's movements so that the counting geometry was constant. Next, the fish were removed from the glass vial and placed in a bag made of fine-mesh net. After excess water was blotted off the fish were weighed to the nearest milligram. After weighing, the fish were removed and the tare weight of the net bag was obtained.

The effective half-life of the isotope in the experimental fish was calculated from the formula

$$T_e = \frac{\log 2}{b},$$

where T_e is the effective half-life and b is the the slope of the calculated regression line of the slower component of the retention curve. Then the relationship between effective half-life and physical half-life is

$$\frac{1}{T_e} = \frac{1}{T_p} + \frac{1}{T_B},$$

where T_p is the physical half-life and T_B is the biological half-life.

MARKING WITH RADIOISOTOPES

Three concentrations of cerium 144 in sea water (0.002, 0.4, and 1.0 μc/ml of sea water) were used to determine the amount of the isotope necessary to mark the fish. After exposure to 0.002 μc of cerium 144 (Experiment 1, Table 1) only three of the six fish tested contained a measurable amount of radioactive cerium, and after 24 hours in nonactive water no measurable activity remained. All fish marked in water containing 0.4 μc cerium (Experiment 2) contained a measurable amount of cerium 144 when removed from radioactive sea water, and could be distinguished from unmarked fish after 33 days in flowing sea water. The fish marked in water containing 1.0 μc of cerium per ml of sea water (Experiment 3) contained a statistically significant (t-test) mean activity relative to control fish after 50 days in flowing sea water. The rate of loss of cerium 144 (Experiment 3) was rapid during the first 10 days after removal from the active water, then remained constant during the next 40 days (Figure 1). Thus, the retention curve may be separated into two components. The slope of the regression line of the slower

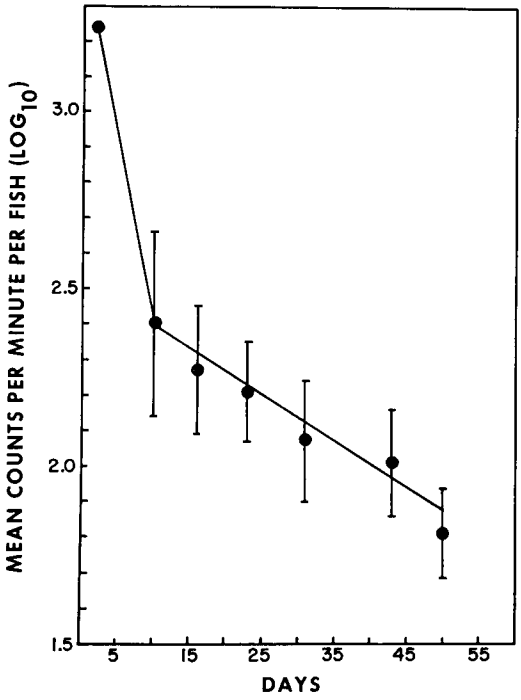

FIGURE 1.—Cerium 144 remaining in flounder held in flowing sea water, after they had been marked in sea water containing 1.0 μc cerium 144 per ml (Experiment 3, Table 1). Vertical lines are one standard deviation.

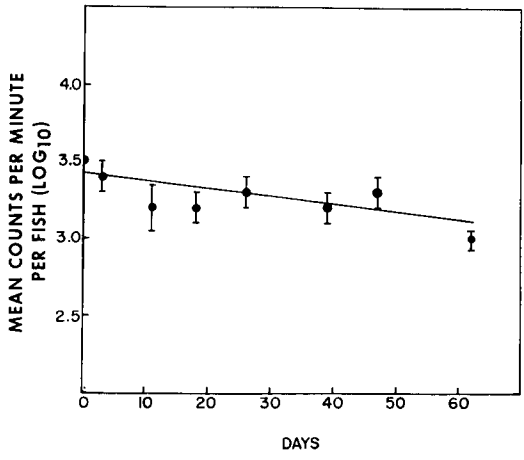

FIGURE 2.—Cobalt 60 remaining in flounder held in flowing sea water, after they had been marked in sea water containing 1.0 μc cobalt 60 per ml (Experiment 6, Table 2). Vertical lines are one standard deviation.

component of the curve (–0.01279) was used to calculate the effective half-life (23 days) and the biological half-life (25 days). Increasing the amount of cerium 144 in the water from 0.4 to 1.0 μc per ml of sea water did not increase significantly the amount of cerium accumulated by the fish in 24 hours.

The amounts of cerium 144 accumulated by the fish from food and from water were compared to determine the best source. Nauplii of the brine shrimp, *Artemia salina*, were made radioactive by placing them in water containing cerium 144 (Experiment 4, Table 1). After 24 hours the nauplii were removed, rinsed in nonactive sea water, and fed to the fish. After the feeding period, contained activity was measured and the fish were placed in nonactive water. A *t*-test comparing the logarithms of the mean counts per minute of cerium 144 accumulation from water (Experiment 2) and from food (Experiment 4) in 24 hours showed that, under the conditions of the experiments, the water source gave counts per minute per fish that were significantly higher at the 1% level.

Flounder were marked in sea water containing 0.01 μc (Experiment 5) or 1.0 μc cobalt 60 per ml (Experiment 6, Table 2). Fish marked in the lower concentration could not be distinguished from controls after remaining in flowing sea water for 26 days, but those marked in the higher concentration retained 29% of the original activity after 62 days in flowing sea water and were easily distinguishable from unmarked fish. The slope of the regression line of the retention curve (Figure 2) was –0.0049; the effective half-life of cobalt 60 was 61 days and the biological half-life 63 days.

To determine whether food or water was the most effective source of cobalt 60 for marking the fish, brine shrimp nauplii were labeled and fed to the fish. The mark produced by feeding the fish nauplii that had been labeled in water containing 0.01 μc per ml cobalt 60 was ineffective. When shrimp labeled in water containing 1.0 μc per ml were fed to fish (Experiment 8), 7% of the original activity remained in the fish after 55 days (Figure 3). The slope of the regres-

TABLE 2.—*Summary of cobalt 60 experiments*

Experiment number	Number of fish marked	Average weight (mg)	Method of marking	Concentration of isotope in water (μc/ml)	Marking time (hours)	Days in flowing sea water	Mean cpm/fish at end of experiment
5	10	54	Water	0.01	48	26	16
6	10	41	Water	1.00	24	62	1,086
7	10	79	Food	0.01	48	3	32
8	10	93	Food	1.00	24	55	531

sion line (Figure 3) was calculated to be −0.017, the effective half-life 17 days, and the biological half-life 17 days.

The difference between the amount of cobalt 60 accumulated from food (Experiment 8) and the amount accumulated from water (Experiment 6) was not significant (*t*-test; 5% level).

MARKING WITH STABLE ISOTOPES

The fish in these experiments were marked in water containing increased amounts of stable cobalt. After 48 hours in this water the fish were removed, rinsed in sea water, and weighed. One-half of the fish were held alive in flowing sea water for use at a later date; the other half were killed by placing them in vials containing 2 ml of 10% formalin solution. The vials were sealed and irradiated in the reactor at North Carolina State University. Groups of five flounders previously held in the following concentrations of cobalt were irradiated for 10 kw hours: (1) control, (2) 0.031, (3) 0.061, (4) 0.122, (5) 0.244, (6) 15.62, (7) 31.3, and (8) 62.5 μg of cobalt per ml of sea water.

After irradiation, fish were returned to the laboratory and held in a lead vault until radionuclides with short half-lives, such as sodium 24, had decayed. The fish then were removed from the vials, rinsed in distilled water, and placed in new plastic containers. The cobalt 60 in the fish was measured in a 512-channel gamma spectrometer.

No difference in cobalt 60 content existed between the unmarked fish and the fish held in 0.244 μg of cobalt per ml before irradiation (Figure 4). There was, however, an obvious difference between the fish marked in water containing 15.62 μg of cobalt per ml and the control fish (Figure 5), indicating that the increased cobalt in the water was sufficient to mark the fish. For a statistical comparison, each marked and unmarked fish was placed in a separate plastic container for measurement of contained activity. The comparison (Dunnett, 1955) indicated that fish marked in water containing 15.62, 31.3, and 62.5 μg cobalt per ml had significantly more cobalt 60 than control fish. Therefore, at the time the fish were removed from the concentrations of cobalt, it was possible to distinguish them from the control fish.

To determine the period of time the mark could be detected, the fish (previously marked in water containing 15.62, 31.3, and 62.5 μg cobalt per ml) were held in flowing sea water for 36 days before irradiation. The procedure described previously was used to determine if the marked fish could be distinguished from the unmarked fish. As before, the differences between the mean values for the marked fish and the mean values for the control fish were significant at the 1% level. On the basis of the activity in each individual, however, it would have been difficult to dis-

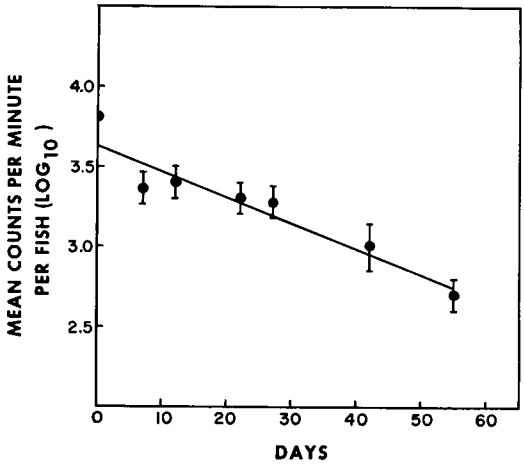

FIGURE 3.—Cobalt 60 remaining in flounder held in flowing sea water after they had been marked by feeding on brine shrimp that had been held in water containing 1.0 μc cobalt 60 per ml (Experiment 8, Table 2). Vertical lines are one standard deviation.

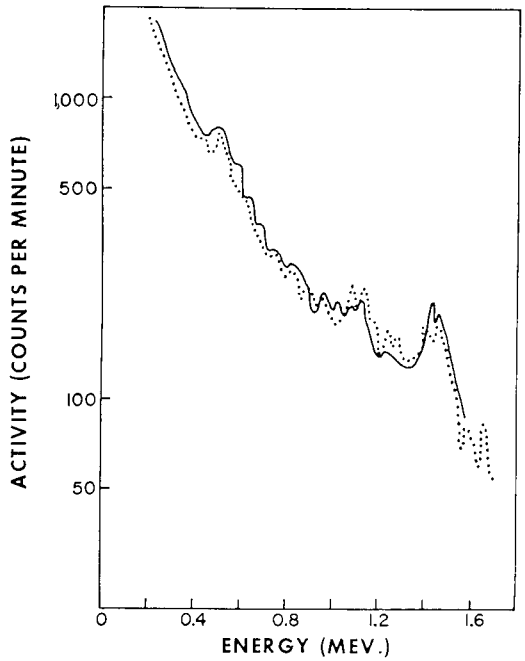

FIGURE 4.—Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. The level of cobalt in flounder was increased by uptake from water containing 0.244 μg cobalt per ml. Solid line represents the "natural" level of cobalt and broken line the increased level.

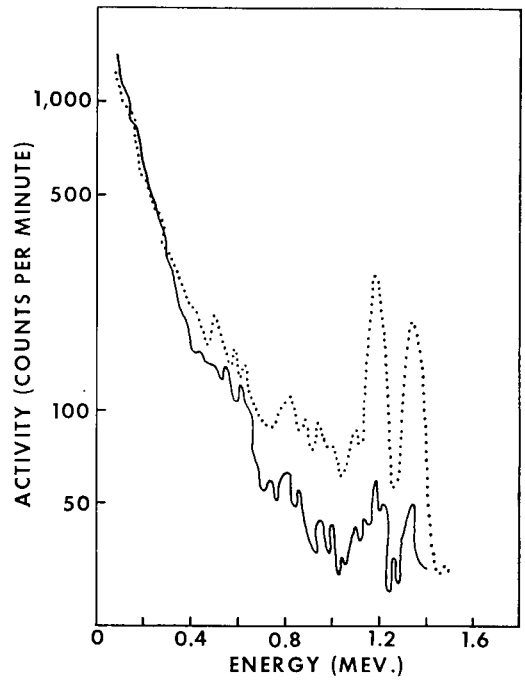

FIGURE 5.—Comparison of gamma spectra of flounder containing a "natural" level and an increased level of cobalt. The level of cobalt in flounder was increased by uptake from water containing 15.62 μg cobalt per ml. Solid line represents the "natural" level of cobalt and broken line the increased level.

tinguish the marked fish in a mixture of marked and unmarked fish under field conditions.

POTENTIAL DANGER TO MAN OF FISH MARKED WITH CERIUM 144 OR COBALT 60

The transfer of radioactivity from fish marked with radioisotopes to other organisms including man must be considered. The number of fish needed to give the maximum permissible body burden of any isotope can be estimated from the amount of the isotope in the fish and the maximum permissible body burden of the isotope, assuming that all of the isotope is transferred from the fish to the consumer. In man the maximum permissible body burdens for occupational exposure to cerium 144 and cobalt 60 are 20 μc and 10 μc, respectively (U. S. National Committee on Radiation Protection and Measurements, 1959). One-tenth of this amount (or 2 μc of cerium 144 and 1.0 μc of cobalt 60) is the permissible level for the general public. Fish marked in water containing 1.0 μc cerium 144 per ml averaged 0.4 μc per fish immediately after they were marked. It would be necessary, therefore, for a person to eat five of these fish to receive the maximum permissible body burden if all the radioactivity were assimilated. Biological loss (excretion) and physical decay further reduce the dose so that 10 days after the fish were marked, it would require 222 fish to reach the permissible burden. The corresponding figures for the cobalt 60 experiments are 50 fish immediately after marking and 100 fish 11 days after marking.

The above figures are an estimation of the maximum amount of activity that theoretically could be transferred from fish to man. Actually, the amount transferred would be much less for the following reasons: (1) By the time the marked flounder reached edible size, only a small part of the activity would remain due to physical decay and biological loss of the isotope; (2) it is not likely that

after a year or two, any person would eat more than one of the marked flounder; (3) a person generally does not eat the entire fish, *i.e.*, radioactivity associated with bones, scales, and intestines would not be transferred to man; and (4) if the marked post-larval fish were not eaten by man but instead passed through several steps of the food chain, activity would be lost at each trophic level, since radioactivity associated with undigestible parts would not be transferred.

CONCLUSIONS ON VALUE OF MARKING WITH RADIOACTIVE ELEMENTS

Both cerium 144 and cobalt 60 were found to be suitable marks for larval fish in these experiments. The effective half-life of cerium 144 obtained from water was 24 days, whereas the effective half-life of cobalt 60 obtained from water was 61 days. Under the conditions of these experiments, the mark (either cerium or cobalt) was retained for a longer period of time when obtained from water. Increasing the concentration of cerium 144 in the water did not produce a measurable increase in the accumulation of this isotope by the fish. Increasing the concentration of cobalt 60 in the water, however, increased accumulation of this isotope.

Significantly increased levels of cobalt were detectable by activation analysis 36 days after fish were marked in sea water containing 15.62, 31.3, or 62.5 μg of stable cobalt. Although the difference between the means of marked and control fish was significant, the actual amount of cobalt per fish was not increased sufficiently to be useful in a field experiment where marked individuals would have to be selected from large numbers of unmarked fish. Thus, the results presented in this paper suggest that marking fish by increasing their stable cobalt content and using activation analysis for detecting the mark is possible, but more of the mark would be required to be incorporated into the fish before the method could be used in the field.

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